Immediate and delayed loading of fixed dental prostheses supported by single or two splinted implants: A histomorphometric study in dogs

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Abstract: To evaluate presumptive differences in osseointegration at implants supporting crowns that are physiologically loaded either immediately or 3 months after installation. All premolars and first molars were extracted bilaterally in six dogs. After 3 months of healing, three implants were installed on the premolar region and two in the molar region in one side of the mandible. Likewise, after another 3 months, five implants were installed in the contralateral side, and impressions were taken bilaterally. Within 48 hours, two single crowns were screwed bilaterally onto two implants in the premolar region, and two splinted crowns reproducing the shape of the first molar were screwed bilaterally onto the implants in the molar region. The mesial implants were used as no-loaded controls. Sacrifices were performed after 3 months, and histological analyses were performed. At the premolar sites, mineralised bone-to-implant contact (MBIC%) was 78.0 ± 4.0% and 70.9 ± 7.9% at the delayed and immediately loaded sites, respectively. This difference was statistically significant. At the control implants, MBIC% was 61.4 ± 14.7% and 63.1 ± 13.1% at the delayed and the immediately loaded sites, respectively. At the molar sites, MBIC% was 79.2 ± 10.9% and 61.1 ± 10.3% at the delayed and immediately loaded sites, respectively. Applying a delayed loading to fixed dental prostheses supported by single or two splinted implants yielded higher proportions of bone-to-implant contact (osseointegration) compared to immediately loaded implants. Moreover, both types of loading protocols yielded a higher rate of osseointegration compared to unloaded implant sites after 3 months following implant installation.

DOI: https://doi.org/10.1111/joor.12612

Originally published at:
Cesaretti, G; Lang, N P; Viganò, Paolo; Bengazi, F; Apaza Alccayhuaman, K A; Botticelli, D (2018). Immediate and delayed loading of fixed dental prostheses supported by single or two splinted implants: A histomorphometric study in dogs. Journal of Oral Rehabilitation, 45(4):308-316.
DOI: https://doi.org/10.1111/joor.12612

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Article type : Original Article

Title: Immediate and delayed loading of fixed dental prostheses supported by single or two splinted implants. A histomorphometric study in dogs.

Running Head: Immediate loading of implants

Article Category: Original Research

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/joor.12612

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Abstract

Objective: To evaluate presumptive differences in osseointegration at implants supporting crowns that are physiologically loaded either immediately or 3 months after installation.

Material and methods: All premolars and first molars were extracted bilaterally in six dogs. After 3 months of healing, three implants were installed in the premolar region and two in the molar region in one side of the mandible. Likewise, after another 3 months, five implants were installed in the contralateral side, and impressions were taken bilaterally. Within 48 hours, two single crowns were screwed bilaterally onto two implants in the premolar region, and two splinted crowns reproducing the shape of the first molar were screwed bilaterally onto the implants in the molar region. The mesial implants were used as no-loaded controls. Sacrifices were performed after 3 months and histological analyses were performed.

Results: At the premolar sites, mineralized bone-to-implant contact (MBIC%) was 78.0±4.0% and 70.9±7.9% at the delayed and immediately loaded sites, respectively. This difference was statistically significant. At the control implants, MBIC% was 61.4±14.7% and 63.1±13.1% at the delayed and the immediately loaded sites, respectively. At the molar sites, MBIC% was 79.2 ±10.9% and 61.1±10.3% at the delayed and immediately loaded sites, respectively.

Conclusions: Applying a delayed loading to fixed dental prostheses supported by single- or two-splinted implants yielded higher proportions of bone-to-implant contact (osseointegration) compared to immediately loaded implants. Moreover, both types of loading protocols yielded a higher rate of osseointegration compared to unloaded implant sites after 3 months following implant installation.

Key words: Animal Experimentation, Immediate Implant Loading , Histology, Implant-Supported Dental Prosthesis, Single-Tooth Implant, Dental Occlusion,

Background

A consensus statement regarding the loading protocol: immediate loading was defined as a restoration placed in function within 2 days[1] or after 3-4 days[2].

In a systematic review,[3] the influence on clinical outcomes of different loading protocols following implant installation was addressed. Articles reporting loadings performed within 1 week of implant installation, between 1 week and 2 months, and after 2 months were included. Moreover, functional and non-functional loadings were assessed. It was concluded that there was no evidence about implant and prosthesis outcomes, as well as bone loss that could be associated with loading protocols.

Nevertheless, in the same review, it was stated that several studies comparing immediate with conventional loading reported similar results regarding implant and prosthesis outcomes.

In an RCT, 30 patients were recruited, and 71 implants were installed.[4] Restorations were performed with 2- or 3-unit bridges, randomly applied either immediately or after 3 months of undisturbed non-submerged healing. After up to 3 years of observation, it was concluded that the survival rate and the radiographic bone levels did not differ between delayed and immediately loaded implants.

The long-term stability of the crestal bone level was furthermore confirmed in a prospective, randomized, split-mouth clinical trial in the posterior mandible, based on up to 15 years of follow-up.[5]

Human studies documenting osseointegration histometrically are relatively sparse. However, a large number of reports on histometric analyses of retrieved implants is available. [6-11] Among these, one study reported data on 17 implants loaded between 4 and 20 years that were retrieved for different reasons from patients and prepared for histometric analyses. [11] All implants were integrated into bone with bone-to-implant contact percentages ranging from 32% to 85%.

Also various animal experiments have attempted to analyze the effect of immediately or delayed loading on osseointegration.[12-21]

In general, the loading in dog experiments has been obtained using a centric occlusion. However, dogs have two different movements that both must be considered physiologically. It should be emphasized that the fourth maxillary premolar has been classified as a “carnassial” tooth with a molar function.[22] In the centric occlusion, the only contacts between maxillary and mandibular arches are on incisors, canines, and molars, while the premolars are not in contact at all with those of the upper jaw. This occlusion is blocked by the anatomical configuration of the temporo-mandibular articulation (ATM), which hampers the dog mandible from performing protrusive and retrusive movements, and by the mandibular canines that are stuck bilaterally between the maxillary canines and third incisors. Posteriorly, the occlusion is supported by a centric contact on the molars. When the dog is chewing, the mandible may gain a lateral position; the first mandibular molar and the maxillary carnassial become the guidance, and the homolateral maxillary canine will be located laterally to the mandibular canine, thus being excluded from contacts and any occlusal guidance.[22] This, in turn, means that the dogs can perform lateral movements. In this lateral position, when the dog bites, the premolars present no contacts, while the mandibular molars gain a more vestibular position that allows the buccal wall of the first mandibular molar to have more effective contact with the lingual wall of the maxillary carnassial (4th premolar). The anterior part of the dentition is used to bite, pull, grab, and tear food, as other carnivores do with their prey. This function is performed in centric occlusion. The molar region is used to cut, chew, and triturate food, such as meat and bones, using both centric and lateral occlusion.

Based on these observations, it is important to elucidate the effects of loading, adopting an occlusion that simulates the anatomical and physiological conditions characteristic for dogs.

Hence, the aim of the present study was to evaluate presumptive differences in osseointegration at implants supporting crowns that are physiologically loaded either immediately or after 3 months from installation.

Material and methods

The research protocol was submitted to and approved by the Ethical Committee of the University of Medical Sciences, School of Dentistry, La Habana, Cuba (#01/2013, approved on May 20, 2013).

Six Beagle dogs approximately 10 kg in weight and approximately 1 year of age were provided by CENPALAB (Centro Nacional para la Producción de Animales de Laboratorio) for the study. The animals were kept in kennels and concrete runs at the University of Medical Sciences field facilities of La Habana, Cuba. The animals had free access to water and were fed moistened balanced dog food.

Clinical procedures

Each surgery was preceded by an injection of atropine 0.02 mg/kg (Mayne Pharma, Napoli, Italia), medetomidine 0.04 mg/kg (Medetor®, Virbac, Glattbrugg, Switzerland), and ketamine-50 5 mg/kg (Liorad, La Habana, Cuba). Isoflurane-Vet® 2.5% (Merial, Merial Tolosa, France) mixed to O_2 at 95% was provided to maintain anesthesia. Local anesthesia was also provided. During the surgery tramadol® 2 mg/Kg (Altadol®, Formevet, Milan, Italy) and amoxicillin® 10 mg/Kg (Convenia®, Pfizer, U.S.A.) were administered.

At the first surgical session, all mandibular premolars and first molars were extracted bilaterally. After 3 months of healing, full-thickness flaps were elevated at one randomly selected site of the mandible, the alveolar bone was exposed, and five titanium implants (Premium®, Sweden & Martina, Due Carrare, PD, Italy), 8.5 mm long and 3.3 mm in diameter, with a neck 0.8 mm high, were installed, with the coronal margin of the rough surface (ZirTi® surface, Sweden & Martina, Due Carrare, PD, Italy) placed at the level of the buccal bony crest. The three mesial implants were installed in the premolar region (#1-3), while the two distal implants were placed in the molar region (#4,5). Healing abutments were affixed onto the implants, and the flaps were sutured to allow a non-submerged healing.

After 3 months, a full-thickness flap was elevated in the opposite side of the mandible, and the same surgical procedures were used. Subsequently, the healing abutments in the other side of the mandible were removed, and transfer copings for taking impressions were applied to the four most distal implants in both sides of the mandible (#2-5). Impressions were obtained in a single-phase using polyvinyl siloxane (Sky light® and Sky medium®, Sweden & Martina, Due Carrare, PD, Italy). Healing abutments were affixed temporarily to all implants, and the flaps at the operated sides were carefully adapted around the healing abutments using single interrupted resorbable sutures (Vicryl® 4–0; Johnson & Johnson, Medical S.p.A., Pomezia, Roma). Within 48 hours, single crowns made of cobalt-chrome were prepared and screwed to the second (#2) and third (#3) implants at both the delayed and immediately loaded sites, with the intent to replace the 3rd and the 4th premolars. On the fourth (#4) and fifth (#5) implants, two cobalt-chrome splinted crowns reproducing the shape of the first molar were screwed onto the two implants with a passive fit, bilaterally (Figure 1AB). Healing abutments were positioned to the most mesial implants (#1) as no-loaded controls.

All crowns were manufactured to reproduce the original anatomy and position of a dog's teeth. Consequently, no occlusal contacts were present at the premolars in both centric and lateral occlusions. The molars were made in such a way to allow contacts at the molars, canines, and incisors in centric occlusion, and between the buccal wall of the maxillary carnassial and the lingual wall of the lower first molar in lateral occlusion. The contacts were checked at all crowns with articulating paper (200 micron, Baush, Nashua, NH, U.S.A.).

Maintenance

Amoxicillin® 20 mg/Kg (Convenia®, Pfizer, U.S.A.) and 2mg/kg tramadol Altadol®, Formevet, Milan, Italy) *per diem* were administered for 5 days after surgery. Daily inspections of the wounds for clinical signs of complications were performed during the first week after the surgeries. Subsequently, inspections and cleaning of the experimental sites were performed twice per week. Sutures were removed after 2 weeks. Occlusal contacts were checked every month, and no apparent occlusal changes were observed.

The animals were sacrificed 3 months after the loading. Sodium heparin 1.000 IU, atropine 0.02 mg/Kg, 1mg/kg of xylazine (Rompun®, Kiel, Germany), and 5 mg/kg of ketamine (Liorad, La Habana, Cuba) were administrated, and the heart was arrested using 25 mEq of potassium chloride i.v. (Aica, La Habana, Cuba). The carotid arteries were subsequently isolated and perfused with a fixative (4% formaldehyde solution).

Histological preparation

Block sections containing one implant each were prepared and placed in 4% formaldehyde. Subsequently, the sections were dehydrated in a series of graded ethanol and then embedded in resin (Technovit® 7200 VLC, Kulzer, Friedrichsdorf, Germany). Each implant was identified inside the block with x-rays and then sectioned in the middle, following its long axis and in a buccal-lingual direction using a diamond band saw in a precision slicing machine (Exakt®, Apparatebau, Norderstedt, Germany). One central section was selected and ground to a thickness of about 50-60Tm using a cutting–grinding device (Exakt®, Apparatebau, Norderstedt, Germany). The histological slides obtained were stained with Stevenel's blue and alizarin red.

Histomorphometric evaluations

The histometric and morphometric analyses were performed with an Eclipse Ci microscope (Nikon Corporation, Tokyo, Japan), equipped with a digital video camera (Digital Sight DS-2Mv, Nikon Corporation, Tokyo, Japan) connected to a computer. The software NIS-Elements D 4.10 was used for measurements (Laboratory Imaging, Nikon Corporation, Tokyo, Japan).

The percentage of mineralized bone and bone marrow in contact with the implant surface was measured at x200 magnification between the most coronal level of osseointegration (B) and the last thread of the implant, both at the buccal and lingual aspects. The morphometric analysis was performed between B and the last thread, up to a distance of 0.4 mm from the implant body. For this purpose, a lattice with squares of 50 μm in dimension was superposed over the tissues at a magnification of x200. Mean values between buccal and lingual aspects were calculated.

Randomization and data analysis

Only the left/ right side of the mandible was randomized (www.randomization.com) for immediate and delayed loading, respectively. The randomization was performed by a person not involved in the surgery (DB). The side to be treated first (delayed loading side) was revealed at the time of the first surgery. Randomization for the unloaded sites was not performed because the most mesial position of the control implant was considered the least suitable for loading due to the small dimensions of this tooth and of the opposite first maxillary premolar. The histological measurements were performed by a person (KAAA) not informed about which side was loaded immediately or delayed.

Mean values and standard deviations (SD) and lower-upper 95% confidence intervals (C.I.) of the differences between immediately and delayed loaded implants were calculated for each outcome variable. Mean values were obtained between the two loaded implants in each side of the mandible, both for the premolar and molar regions. The main variable was the percentage of mineralized bone-to-implant contact at the immediately and delayed loaded implants. Differences between the immediately and delayed loaded implants were analyzed with IBM SPSS statistics software (IBM Inc., Chicago, IL, USA) using the Wilcoxon signed rank test. The level of significance was set at α=0.05. As an exploratory aim, differences were also assessed between the loaded implants and the corresponding control implants of the premolar group, i.e., those located in the same half-mandible.

Results

Mineralized bone-to-implant contact and bone density at immediately and delayed loaded implants at the premolar and molar regions are presented in Table 1. Mineralized bone-to-implant contact and bone density at the control sites are presented in Table 2.

Premolars sites

Mineralized bone-to-implant contact (MBIC%) was 78.0±4.0% and 70.9±7.9% at delayed (Figures 2A, 3AB) and immediately loaded (Figures 2B, 4AB) sites, respectively. This difference was statistically significant (p=0.046; C.I. 2.8%; 11.4%). The control implants at the delayed sites (Figure 5A) presented a MBIC% of 61.4±14.7%; the difference with the corresponding loaded sites was statistically significant (p=0.028; C.I. 6.9%; 26.3%). The unloaded implants of the immediately loaded sites had a MBIC% of 63.1±13.1%; the difference with the corresponding loaded sites was not statistically significant (p=0.116; C.I. 1.0%; 14.7%).

Bone density % was 76.0±9.1% and 71.1±11.6% at the delayed and immediately loaded implants (p=0.249; C.I. -3.7%; 13.5%). The percentages at the respective unloaded implants were 72.8±13.1 and 67.9±15.7%. No statistically significant differences were found.

Molars sites

MBIC% and bone density % were 79.2±10.9% and 75.0±9.9% at the delayed loaded sites (Figure 5B, 6A) and 61.1±10.3% and 63.7±6.0% at the immediately loaded implants (Figure 6BC), respectively. Both differences were statistically significant (MBIC% p=0.028; C.I. 11.9%; 24.3%) (bone density % p=0.031; C.I. 3.8%; 18.8%).

Discussion

The aim of the present study was to evaluate presumptive differences in osseointegration at implants supporting fixed dental prostheses and loaded either immediately or after 3 months following installation. Higher mineralized bone-to implant contact and bone density were observed at the delayed implants compared to the immediately loaded implants. Likewise, the bone-to-implant contact at unloaded control implants was significantly lower than that of the loaded implants (both immediately or delayed loaded).

The effects of loading on osseointegration was histologically documented in human studies and contradictory results were reported.[6,7,23,24]

In a histomorphometrical analysis of retrieved implants, [24] 29 implants with different configurations and surfaces were used. The implants were immediately loaded and retrieved after 2 to 10 months of function, and studied histologically. A mean bone-to-implant contact percentage of 66.8% was found, confirming that immediate loading may yield predictable osseointegration.

In another study, four volunteers underwent oral rehabilitation by means of implants.[6] Each patient received one additional implant. Two of these additional implants were immediately loaded and two were left unloaded. The implants were retrieved with trephines after either 4 or 8 weeks. After 4 weeks of healing, the bone-to-implant contact was 65.6% and 54.7% at the loaded sites and unloaded sites, respectively. After 8 weeks, the bone-to-implant contact was 76.2% at the loaded sites and 62.3% at the unloaded sites. It has to be realized that, even though osseointegration was higher at the loaded sites compared to the unloaded sites, the sample size in that study was only one for each comparison.

A positive effect of loading was also reported in another study in which five patients received nine additional implants during implant treatment.[23] Two implants were loaded immediately and seven were loaded after 2 months of healing. These implants were retrieved after 5-9 months of function. Higher bone-to-implant contact and bone density were found at the immediate implants compared to the delayed loaded implants.

An effect of load on osseointegration was however not confirmed by another human study.[7] In this study, each of the 13 volunteers recruited received two implants, one immediately loaded and the other left unloaded. The recipient sites were prepared with either drills alone or drills followed by the use of osteotomes to finalize the recipient sites. Biopsies were collected after 1 and 3 months. No statistically significant differences could be found between loaded and unloaded sites after either 1 or 3 months.

Similar outcomes have been reported by another human study, in which–two mini-implants were installed in 16 volunteers.[25] After 2 months of healing, one implant was loaded while the other was left unloaded. After 2 more months of healing, biopsies including the mini-implants were retrieved in 10 patients and histologically analyzed. No statistically significant differences were discovered between the loaded and unloaded sites, neither for the total mineralized bone in contact with the implant surface (86.8% and 84.6%, respectively) nor for the bone density (76.8% and 74.1%, respectively).

An high degree of osseointegration (mean 52.9%) was also found at titanium transitional implants, with a machined surface, that were used to fix provisional restoration.[26]

Several experiments evaluating the effect of loading on osseointegration also have been performed in various animals models, such as monkeys,[13, 27-31] dogs,[12; 14-18; 20, 21, 32-36] pigs[37-39] and rabbits.[40] Some experiments evaluated osseointegration when a physiologic load was applied.[13,14,20,21,31,33,37-39] Other experiments applied an overload at the test sites,[27,29,30,34] or a non-axial load[32] or a lateral load.[12]

A similar protocol to that applied in the present study was adopted in an experiment in nine monkeys. Immediate and delayed loaded sites, as well as unloaded sites, were studied. Implants were installed 3 months after the extraction of the second premolar, and of the first and second molars, in both sides of the mandible.[13] In the control group, the implants were left to heal submerged for 3 months. In the delayed loaded group, the implants were left to heal submerged for 3 months, and then were loaded for another 3 months. In the third group, the implants were loaded immediately after installation and left to function for 3 months. Osseointegration was found to be slightly higher in the delayed loaded group (67.9%) compared to the immediately loaded group (64.2%). However, bone density within the thread areas was found to be higher at the immediately loaded (76.9%) compared to the delayed loaded implants (65.4%).

To increase the possible effect of load on osseointegration, premature contacts were incorporated in some studies.[27,29,30,34]

In a study in six Labrador dogs,[34] all premolars were extracted, as well as the first and second molars, which eliminated their support in centric occlusion. After 3 months of healing, four implants were installed on each side of the mandible. After another 6 months, gold crowns, fabricated with an increased vertical dimension to ensure premature contacts, were applied on one side of the mandible, while no load was applied at the control sites. After 8 months of healing, mineralized bone-to-implant contact was 73% at the control implants and 74% at the loaded implants, so an effect of the load on osseointegration could not be confirmed.

In other studies, contacts in centric occlusion were obtained.[14, 20] In an experiment in six Beagle dogs,[14] all mandibular premolars were extracted, while the molars were left in situ bilaterally so that a distal support in centric occlusion was ensured. After 3 months of healing, four implants were installed in each side of the mandible in a submerged fashion. After another three months, abutment connections were performed while the maxillary canines and premolars were prepared for prosthesis.

Fixed partial dentures (FDPs) were cemented at the maxillary canine-premolars while FDPs were screwed to the three posterior implants, bilaterally. A “flat-to-flat” occlusal contact between the mandibular and maxillary FDPs were obtained. The most anterior implant was left unloaded on both sides of the mandible. After 10 months of functions, biopsies were obtained. A higher bone-to-implant contact was observed at the loaded sites compared to the unloaded controls, confirming that a longstanding functional load may influence the quality of osseointegration.

In another experiment in 6 Labrador dogs[20] including a shorter period of function compared to the previous discussed studies, all mandibular premolars and the first molars were extracted, while the second molars were maintained bilaterally, preserving the distal support in centric occlusion. Two implants were installed 3 months after tooth extraction in both sides of the mandible in the premolar region using insertion torques either of about 30 Ncm or >70 Ncm. Tooth preparation of the maxillary second and third premolars of the loaded sides was carried out. Within 24 hours, single crowns were affixed to the implants, while a bridge was cemented to the maxillary premolars at the immediately loaded sites. Contacts in centric occlusion between the mandibular and maxillary prosthesis were obtained. At the control sites, the implants were left unloaded. Bone-to-implant contact and bone density were found to be higher at the loaded sites compared to the unloaded sites, confirming a positive effect of load on osseointegration. At the loaded sites, bone-to-implant contact was 83.1% and 72.2% at the implants installed with insertion torques of 30 Ncm and >70 Ncm, respectively. The respective percentages at the unloaded sites were 72.4% and 68.3%.

It should be noted that, in the above-discussed experiments,[14, 20, 34] only the centric occlusion was considered while the function in lateral occlusion was disregarded. This means that it is impossible to confirm any contact in the lateral position during the chewing procedures in any of the three studies. This uncertainty may explain the different results in confirming the positive effect of load on osseointegration.

In the present study, the prostheses on implants were designed and finalized according to the anatomy and physiology of teeth and occlusion in the individual dog.[22] Following these principles, the mandibular premolars were made in such a way that they were not in contact with those of the upper jaw in centric occlusion, and their unmodified anatomy did not to interfere with the function of the tongue. The mandibular first molar was designed to have an occlusal contact with the upper carnassial (fourth premolar) in the centric occlusion, and at the same time allow contacts between second molars, canines, and incisors of the two jaws. Moreover, in the lateral occlusion, the design of the crowns provided a first-molar guided occlusion that also allowed the dog to chew food.

The prosthesis was delivered within 48 hours, and not immediately after implant installation, according to the consensus statement regarding the loading protocol.[1] This, in turn, meant that there was no possible influence on healing of a functional or a progressive loading applied immediately after implant installation.[41]

In conclusion, applying a delayed loading to fixed dental prostheses supported by single- or two-splinted implants yielded a higher proportion of bone-to-implant contact (osseointegration) compared to immediately loaded implants. Moreover, both types of loading protocols yielded higher osseointegration compared to unloaded implant sites after 3 months after implant installation.

The lack of control in the function in lateral occlusion in a dog model may fail to reveal a positive effect of load on osseointegration.

Disclosure/Acknowledgements

This study has been supported by a grant from Sweden & Martina SRL, Due Carrare, Padova, Italia, by ARDEC Academy, Ariminum Odontologica SRL, Rimini, Italia, and by the Clinical Research Foundation (CRF) for the Promotion of Oral Health, Brienz, Switzerland. We acknowledge the contribution of Prof. Joaquin Urbizo Velez (Faculty of Dentistry of La Habana, Cuba) for the histological processing.

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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Table 1. Mineralized bone-to-implant contact percentage (MBIC %) and bone density % at the delayed and immediate loaded sites. Mean and ±SD and Median (25%; 75% percentiles).

	PREMOLARS		**MOLARS**	
	MBIC %	**Bone density %**	**MBIC %**	**Bone density %**
DELAYED LOADING	78.0 ±4.0*#	76.0 ±9.1	79.2 ±10.9*	75.0 ±9.9*
	78.8 (76.2; 80.1)	77.9 (68.2; 82.9)	83.9 (81.1; 84.8)	73.3 (68.3; 82.3)
IMMEDIATELY LOADED	70.9 ±7.9*	71.1 ±11.6	61.1 ±10.3*	63.7 ±6.0*
	69.2 (64.1; 76.6)	68.7 (67.1; 70.8)	64.9 (55.1; 69.2)	63.0 (60.5; 64.4)

*** = p<0.05 between delayed and immediately loaded.**

 # = p<0.05 between loaded sites and the respective control implants.

Table 2. Mineralized bone-to-implant contact percentage (MBIC %) and bone density % at the control sites. Mean ±SD and Median (25%; 75% percentiles).

	PREMOLARS	
	MBIC %	**Bone density %**
DELAYED LOADING	61.4 ±14.7#	72.8 ±13.1
	59.8 (49.6; 73.3)	75.0 (70.2; 76.0)
IMMEDIATELY LOADED	63.1 ±13.1	67.9 ±15.7
	68.3 (51.4; 72.4)	70.8 (56.1; 78.2)

*** = p<0.05 between delayed and immediately loaded.**

 # = p<0.05 between loaded sites and the respective control implants.

Legends

Figure 1. Cast models. (A) Crowns made of cobalt-chrome replacing 3rd and 4th premolars and two splinted crowns replacing 1st molar. (B) Centric occlusion from behind.

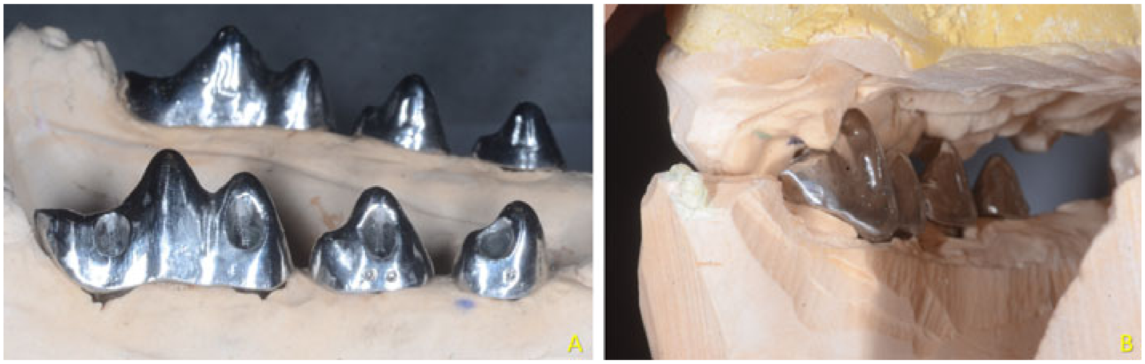
Figure 2. Ground sections representing the healing at (A) delayed loaded and (B) immediately loaded implants supporting single crowns located in the premolar regions. The photomicrographs were original grabbed at x20 magnification. Toluidine blue stain.

Figure 3AB. Ground sections representing the healing at delayed loaded implants in the premolar sites. Mature bone, presenting several regions of remodeling processes, is surrounding the implant surface. The photomicrographs were original grabbed at x100 magnification. Toluidine blue stain.

Figure 4AB. Ground sections representing the healing at immediately loaded implants in the premolar sites. Mature remodeled bone and marrow spaces surrounding the implant surface. The photomicrographs were original grabbed at x100 magnification. Toluidine blue stain.

Figure 5. Ground sections representing the healing at (A) a control implant installed in the premolar region and (B) at a delayed loaded implant contributing to support a crown in the molar region. The photomicrographs were original grabbed at x20 magnification. Toluidine blue stain.

Figure 6. Ground sections representing the healing at implants in the molar region. (A) Delayed loaded implant surrounded by mature dense bone. (B,C) Immediately loaded implant in the molar sites with higher content of marrow spaces. The photomicrograph A was original grabbed at x100 magnification. The photomicrographs B and C were taken at x100 magnification. Toluidine blue stain.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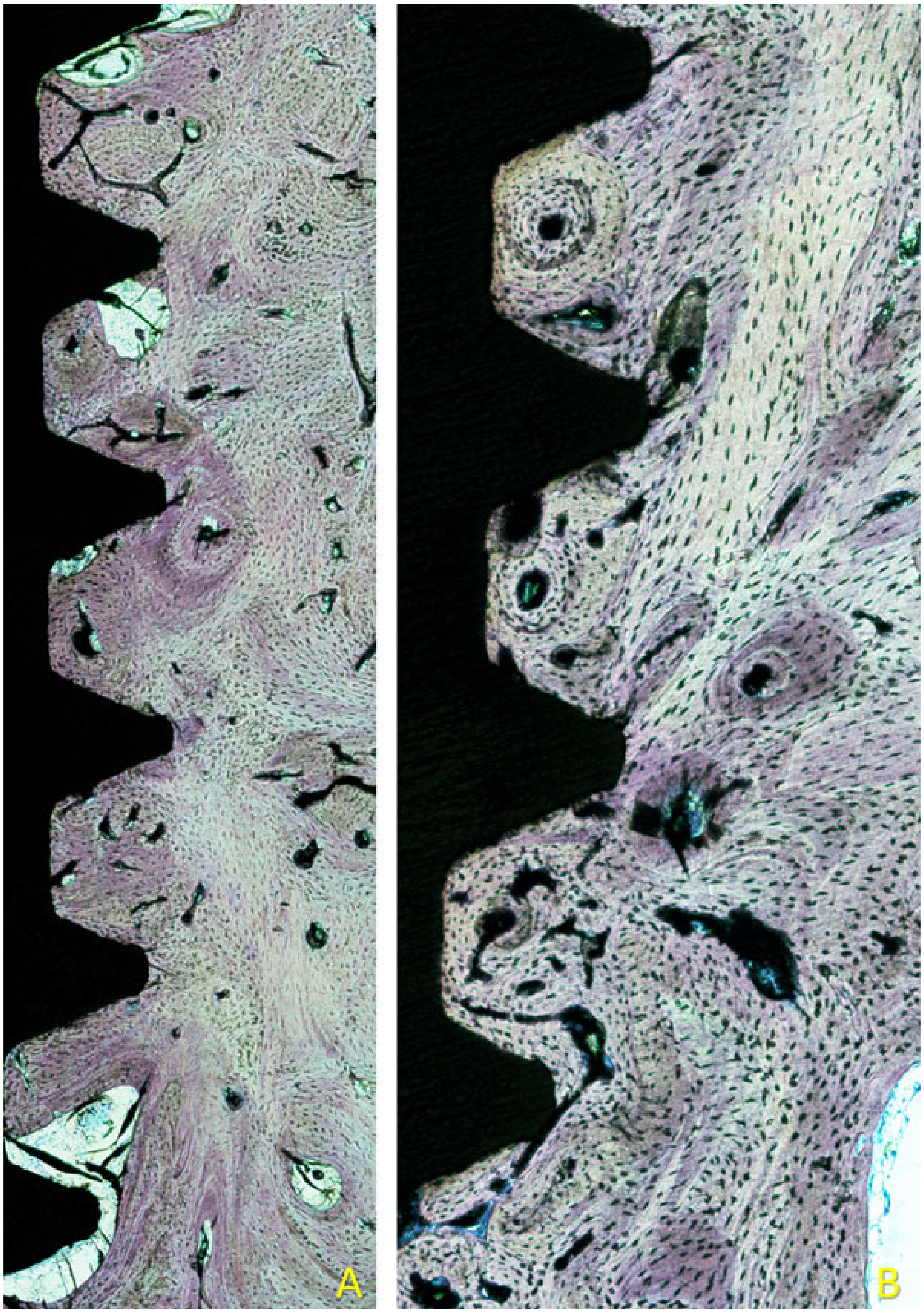
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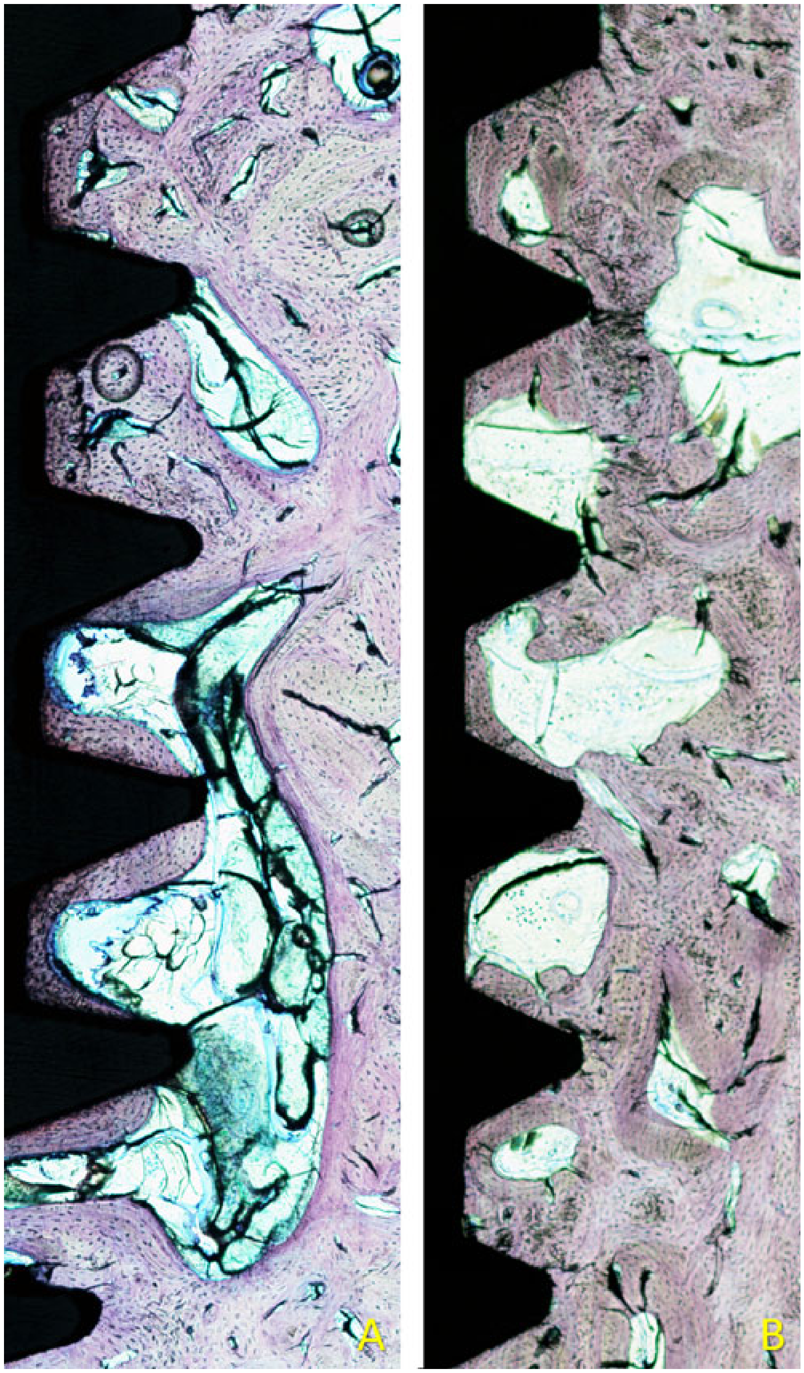
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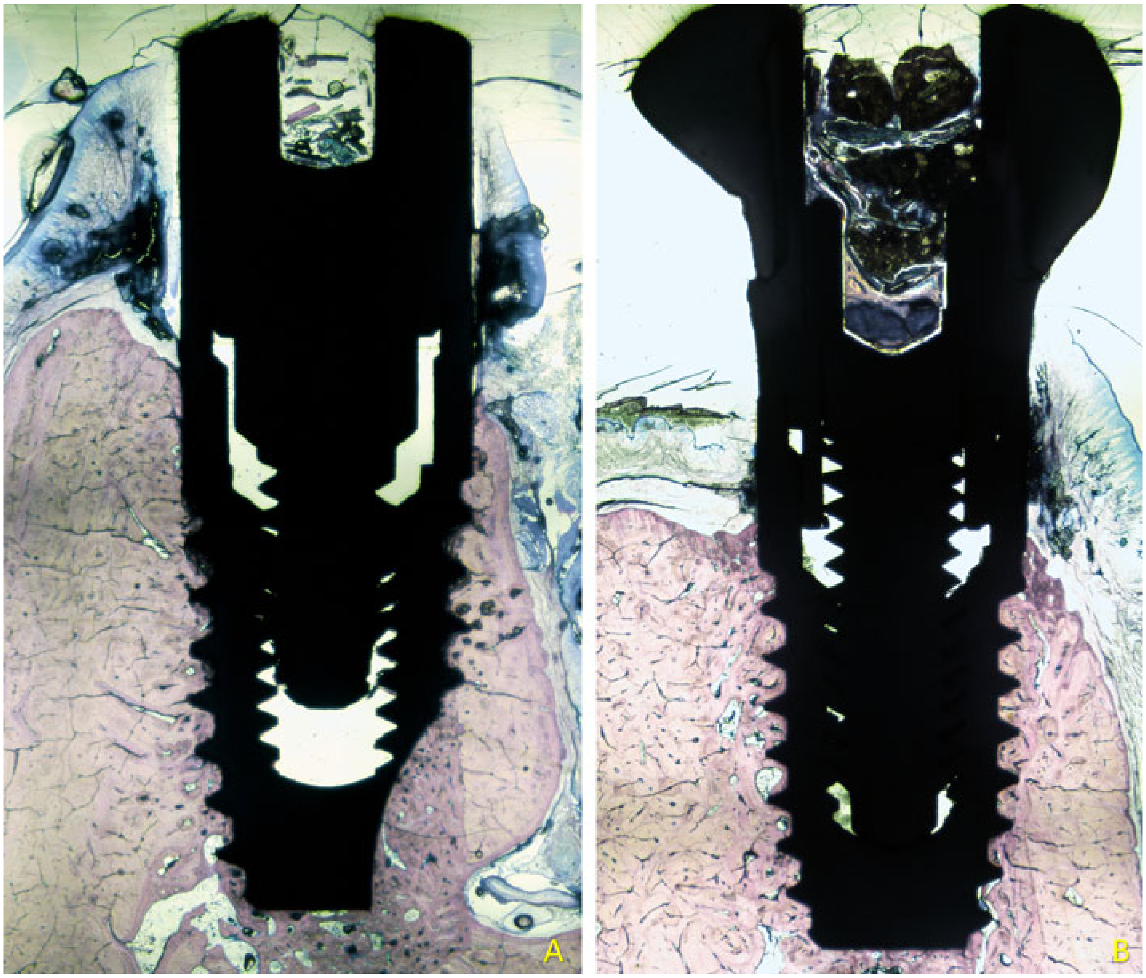
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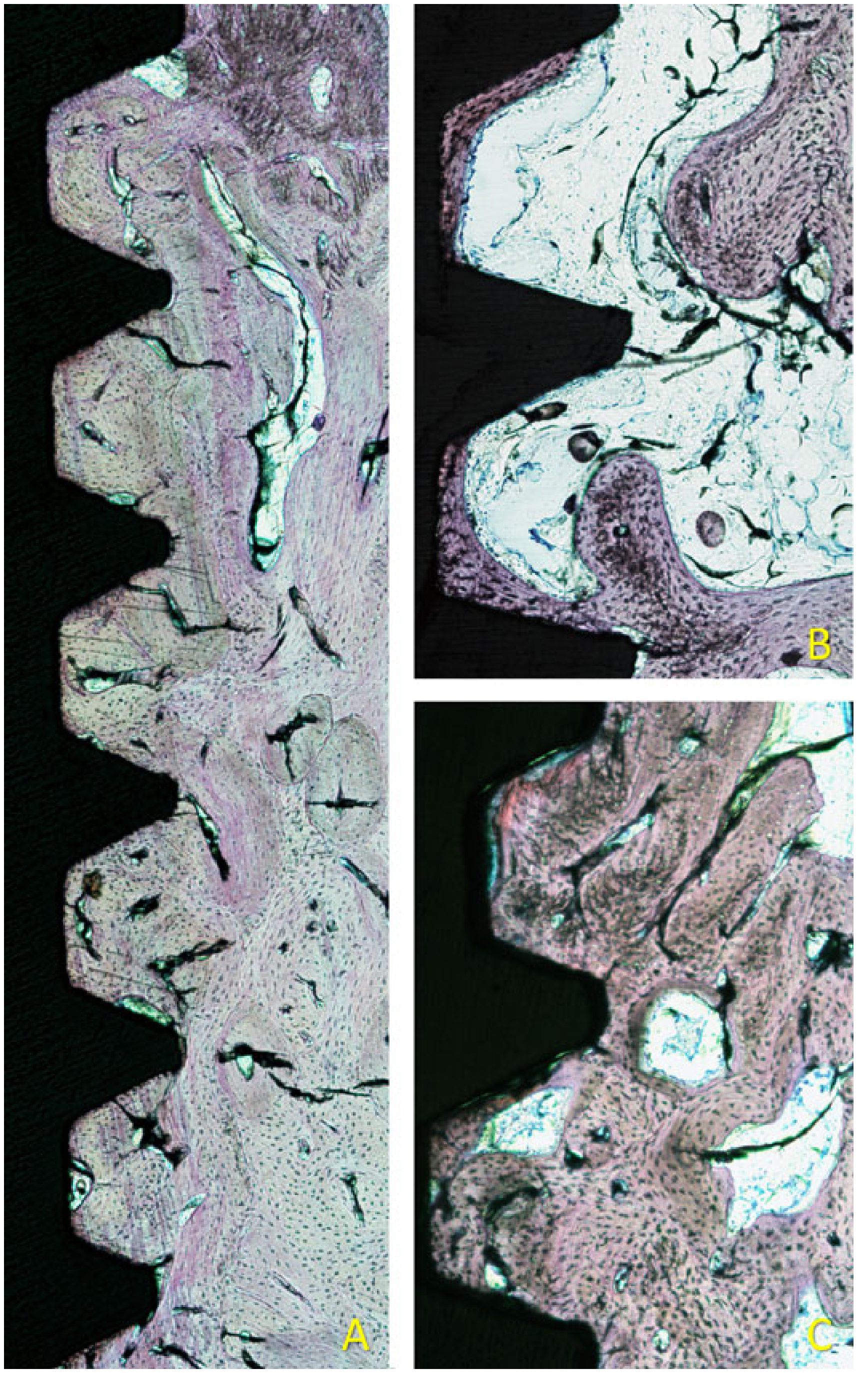
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